American Fern Journal 90(1):32–41 (2000)

SEM Studies on Vessels in Ferns. 19. *Marsilea*

EDWARD L. SCHNEIDER and SHERWIN CARLQUIST
Santa Barbara Botanic Garden, 1212 Mission Canyon Road, Santa Barbara, CA 93105

ABSTRACT.—Tracheary elements from roots and rhizomes of *M. drummondii, M. quadrifolia,* and *M. vestita* supplement the work of other authors by showing features of *Marsilea* vessels not previously reported or not commonly reported. Pit dimorphism (wide pits lacking pit membranes alternating with narrow pits covered with pit membranes) form perforation plates in some vessel elements. Scalariform perforation plates with perforations like lateral wall pitting in size are common, and have not been previously reported in *Marsilea* by workers using light microscopy; further SEM studies may reveal such perforation plates to be more common in the family than presently thought. Circular to oval pits arranged in alternate, opposite, and transition fashion are characteristic of some vessels in the genus, as are tracheary element facets with few or no pits. The occurrence of perforation plates with few bars (found also in xeric and boreal ferns) and simple perforation plates in *Marsilea* may represent a capability for rapid uptake of water in habitats with relatively short periods of water availability.

Marsileaceae have been explored more thoroughly than other fern families for presence and morphology of vessels. Following the initial reports of vessels in roots of *Marsilea* by White (1961, 1962), Mehra and Soni (1971) confirmed presence of vessels in *Marsilea* roots but did not find them in rhizomes of the genus. Tewari (1975) reported vessels in roots of *Regnellidium.* Bhardwaja and Baijal (1977) reported vessels in roots of nine species of *Marsilea,* but in rhizomes of only two *Marsilea* species. Loyal and Singh (1978) observed vessels in roots of seven species of *Marsilea,* and also found vessels in the nodal regions where roots and petioles join the rhizome, but not in internodal regions of the rhizome. Bhardwaja and Takker (1979) observed only tracheids in xylem of *Pilularia.* Details on occurrence of vessels in *Marsilea* were added by Johnson (1986); Sharma (1988) cites the work prior to Johnson's on vessels in Marsileaceae, but does not add original observations.

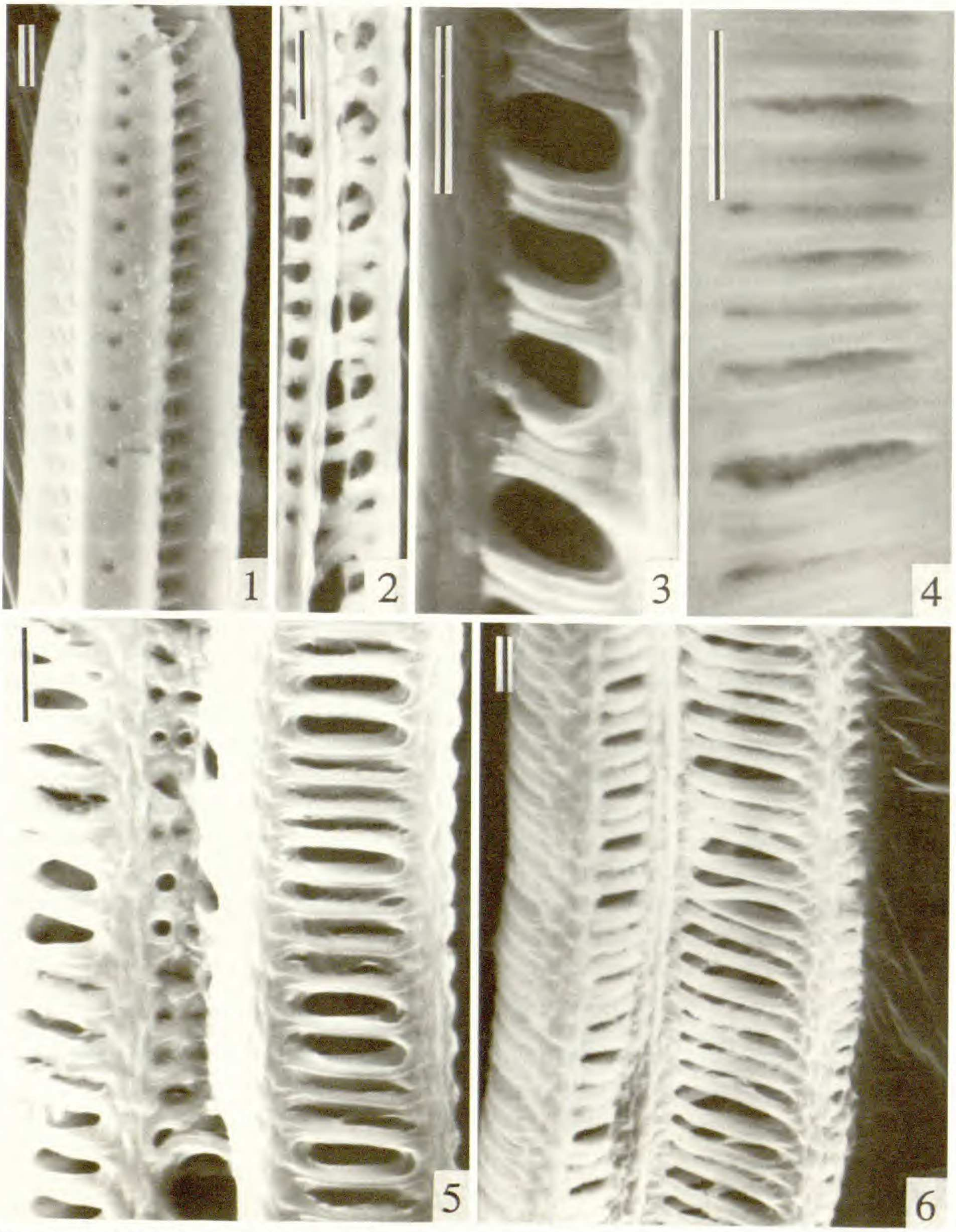
Note should be taken that all of the studies cited above were performed with light microscopy. Although vessels with well-defined perforation plates consisting of few perforations may be observed with reasonable accuracy with light microscopy, observation of fern vessels with SEM (scanning electron microscopy) seems a procedure that can add more information and is therefore of value. In most of the fern families studied in our papers, we have described vessel elements in which perforation plates are very similar to, or identical to, lateral walls of tracheary elements, and differ in absence of primary walls in the perforations, which are like lateral wall pits in size and shape; the distribution of perforations as seen by SEM is also distinctive and diagnostic, and not at all random. We believe SEM is valuable in identifying such probable perforations. In some carefully observed preparations utilizing particular stains, light microscopy has been used to establish presence or absence of primary walls in possible perforation plates in vessel elements. SEM, however, clearly reveals presence of cell walls, which reflect the electron beam. Presence of primary walls and of perforations can be established accurately by means of transmission electron microscopy (TEM). Ultimately, one would wish for observations by all kinds of microscopy, as well as the utilization of particles that can be transmitted through perforations but not through intact pit membranes.

We believe that our results with SEM are accurate indications of vessel presence in ferns because the macerations (and in some instances, sections) in our studies reveal pit membranes with few artifacts. Some pit membranes are ruptured by handling, but removal of all pit membranes on an end wall while pit membranes are intact on adjacent lateral walls cannot, in our opinion, be attributed to the maceration process. Moreover, we frequently find at ends of perforation plates pit membranes that are transitional to perforations by virtue of thinness and presence of porosities or even minimal weblike or strandlike remnants of primary walls. Such incipient perforations can also be found on end walls that have no perforations devoid of pit membranes. By eliminating from consideration all instances we could ascribe to pit membrane absence that might represent artifacts induced by preparation methods, we believe that our reports of perforation plate presence are reliable.

The studies on xylem of Marsileaceae cited above report vessel presence in rhizomes much less frequently than in roots. However, our studies on vessels in ferns have found that in any species in which vessels could be demonstrated in roots, they could also be demonstrated in rhizomes. On account of this finding, we were motivated to search for vessels in rhizomes of *Marsilea*. In fact, the sheaths of fibers around the steles of *Marsilea* roots provided severe difficulties in isolating tracheary elements for observation by SEM, whereas rhizome steles yielded somewhat more easily to the maceration process. These maceration difficulties, greater than those we have encountered in any other fern families in our series of papers, have limited the number of vessel elements we could observe clearly. Consequently, the observations we report here should not be taken as representative of kinds of vessels in these species. Our observations do show the presence of tracheidlike vessel elements in which scalariform perforation plates resemble scalariform lateral wall pitting. These have not been reported by earlier authors and thus we add to the picture of perforation plates in *Marsilea*.

Materials and Methods

Species studied and the sources of collections are as follows: *M. drummondii* A. Braun, cultivated at the University of California Botanic Garden, Berkeley (74.0212); *M. quadrifolia* L., cultivated by San Marcos Growers, Santa Barbara, California; *M. vestita* Hook & Grev., cultivated in the Biological Sciences greenhouses, University of California, Santa Barbara. Voucher specimens prepared from these plants have been deposited in the herbarium of Santa Barbara Botanic Garden. Portions of roots and rhizomes were fixed and stored in 50% aqueous ethanol. Macerations were prepared by means of Jeffrey's Fluid (Jo-



FIGS. 1–6. SEM photographs of tracheary elements from macerations of roots (Fig. 1) and rhizomes (Figs. 2–6) of *Marsilea drummondii*. 1) Element with scalariform facet (right), facet with small circular pits (center), and facets with few, sparse pits (left). 2) Slender tracheary element with facets bearing perforations. 3) Portion of perforation plate in which perforations are large, the result of pit dimorphism. 4) Lateral wall with pits that have thin, incipiently porose pit membranes. 5) Two adjacent elements with apparent groups of perforations separated from each other by pits; some pits in the facet, left of center, are arranged in opposite fashion. 6) Two adjacent elements; wide scalariform facet (right of center) is a perforation plate, with a few pits interspersed

hansen, 1940), stored in 50% ethanol, spread onto aluminum stubs, air-dried, sputter-coated, and observed with a Bausch and Lomb Nanolab 200 SEM at 15 KV.

RESULTS

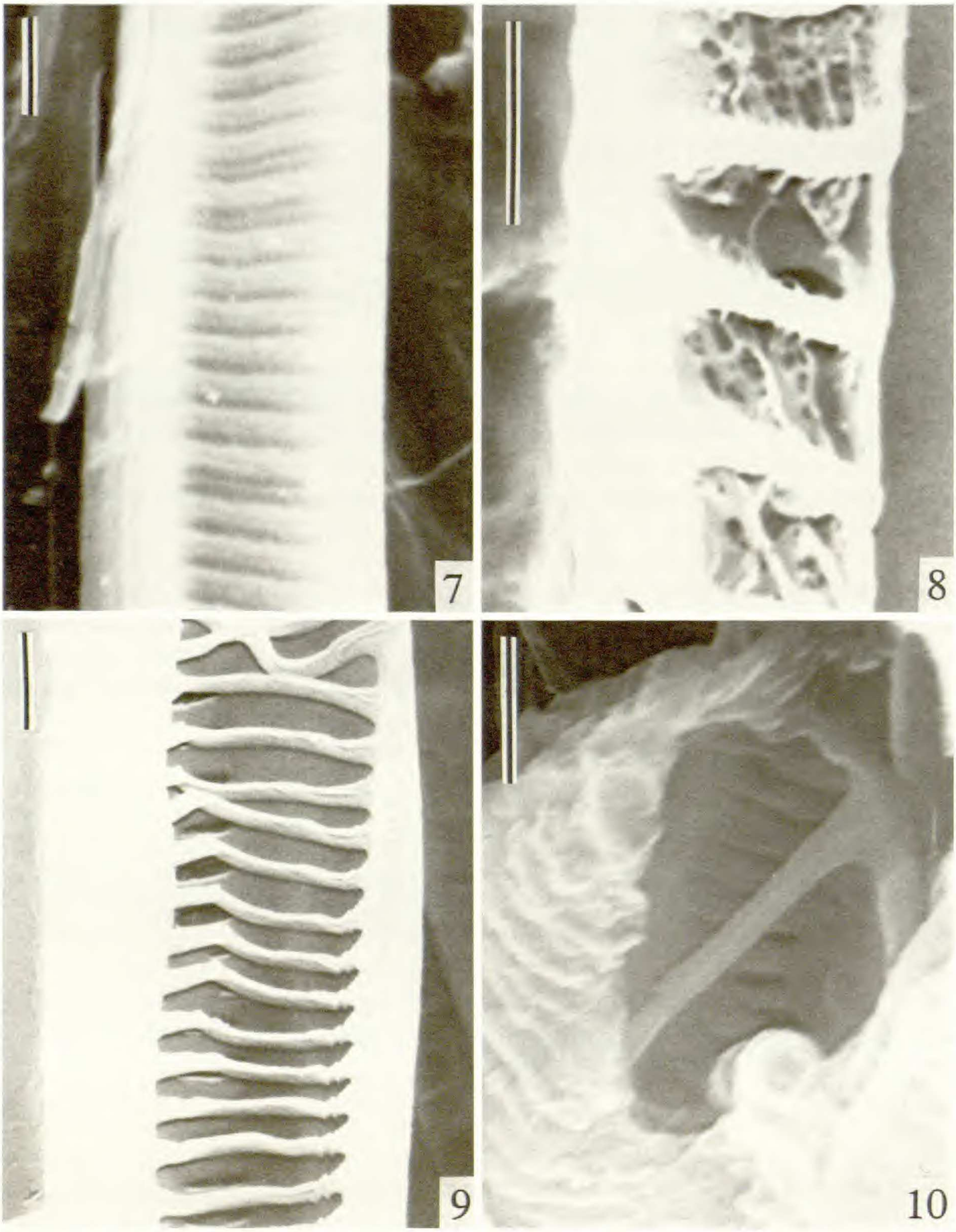
Marsilea drummondii root xylem proved unusually difficult to isolate by means of maceration. The root tracheary element shown in Fig. 1 illustrates scalariform lateral wall pitting, at right. The facet at the center of the photograph is so narrow that the pits are circular in outline, whereas the facet at left bears pits that are few and small.

Rhizome tracheary elements of *M. drummondii* (Figs. 2–6) were more readily recovered by maceration and proved to bear abundant and diverse perforations. In Fig. 2, pit membranes are absent from all of the facets of a slender tracheary element, and perforations are of various sizes. The phenomenon of dimorphic pits is illustrated in the facet shown in Fig. 3; wide perforations alternate with several pits compressed parallel to the long axis of the tracheary element. The compressed pits are so narrow that one cannot readily observe pit membranes on them. Pit membrane remnants certainly are absent on the wide perforations. Pit membranes are present on the scalariform pits of the lateral wall illustrated in Fig. 4. We cannot certify that pores are present in these pit membranes, but thin areas are discernible in the pit membranes. In a tracheary element shown in Fig. 5 (facet at right), on the other hand, pits that apparently lack pit membranes (and would therefore be perforations) are present in groups, and these groups alternate with groups of several pits that bear intact pit membranes (some membranes torn by handling). If this interpretation is valid and does not represent a condition caused by artifact formation, various facets of the tracheary elements illustrated have what one can regard as intermittent perforation plates. This situation is also present in the pair of adjacent tracheary elements in Fig. 6, although perforations are somewhat more abundant in the tracheary element at right. In the tracheary element at left in Fig. 6, the left facet consists of pits with intact membranes, and the right facet bears, above, narrowed pits and slightly widened perforations alternating with each other, an example of pit dimorphism.

Tracheary elements from roots of *M. quadrifolia* are illustrated in Figs. 7–10. The lateral wall in Fig. 7 bears pits with intact pit membranes, and is noteworthy only in that the pits are irregularly spaced: a few pits appear in close pairs. In Fig. 8, pit membrane remnants of an incipient perforation plate bear striking pores or weblike strands of primary wall material can be seen. Tears due to handling may be seen in the pit membrane remnants, but these can easily be detected and the porose nature of the pit membranes is clear. In

←

among the perforations; facet left of center shows, above, alternation of perforations and narrow pits (pit dimorphism); facet at left is composed of scalariform pitting. Scales in all figures = 5 μm.



FIGS. 7–10. SEM photographs of tracheary elements from roots of *Marsilea*. 7) Facet bearing narrow pits, some of which are in close pairs. 8) Facet in which pit membrane remnants are markedly porose; some tearing present but most areas are intact. 9) Scalariform perforation plate portion; bars are narrow, perforations wide, and thus the perforations differ in width from lateral wall pits (compared with Fig. 7). 10) Perforation plate bearing a single bar. Scales in all figures = 5 μm.

Fig. 9 a scalariform perforation plate with perforations wider (with respect to the element axis) than pits (compare with Fig. 7) is illustrated. In Fig. 10 an end wall perforation plate traversed by a single bar is shown. We did not with certainty observe any tracheary elements with simple perforation plates.

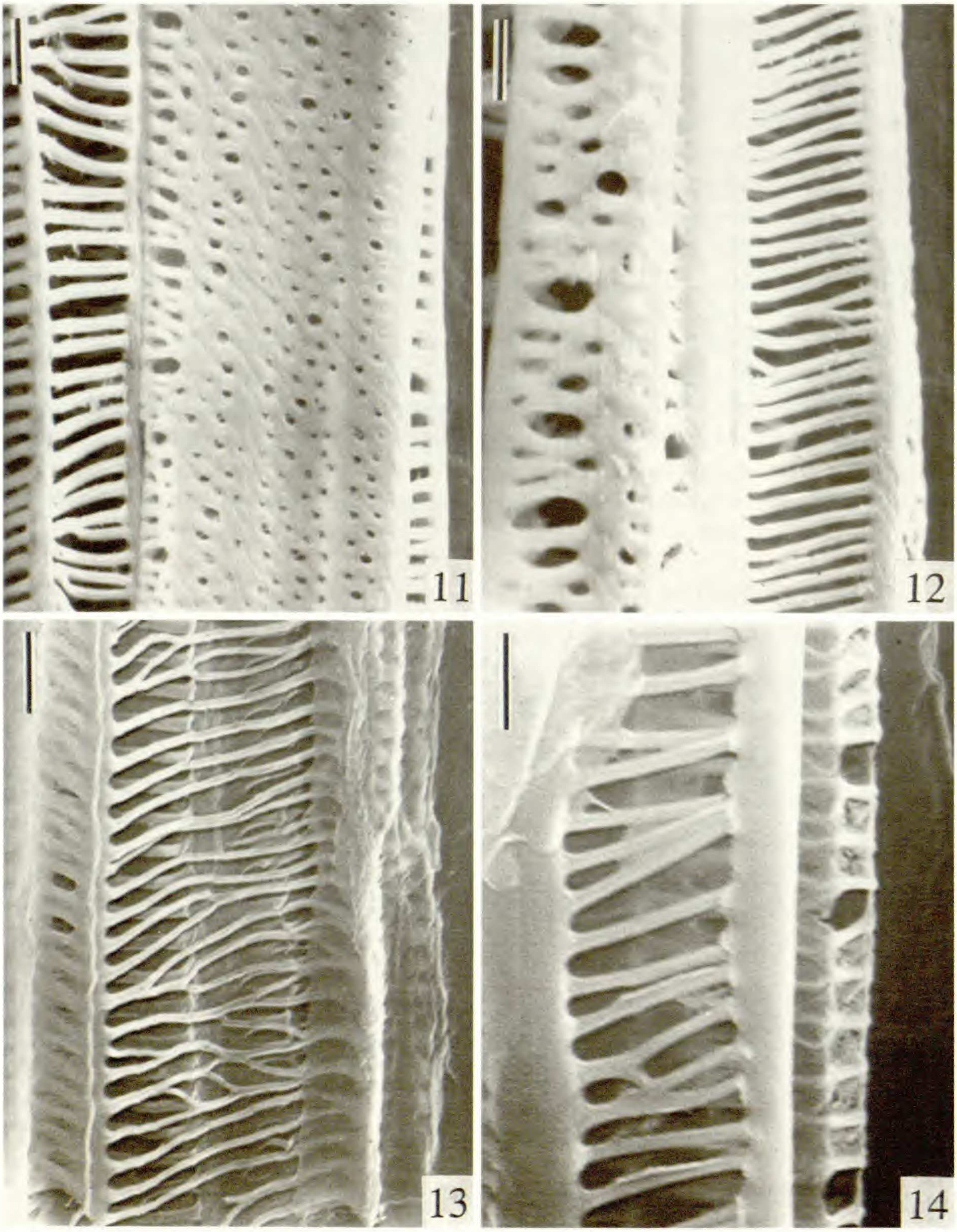
Rhizome tracheary elements of *M. quadrifolia* are relatively wide in diameter, and at least some have well-defined perforation plates (Figs. 11–14). Scalariform perforation plates are illustrated in Fig. 11, left, and Fig. 12, right; the perforations contain pit membrane remnants that do not appear to have resulted from handling. In both Fig. 11 and Fig. 12, alternately arranged circular to oval pits cover other facets. Pitting of this type was reported in drawings of Loyal and Singh (1978, Figs. 35 and 36). However in the tracheary elements in our figures, there is wide diversity in pit size on a single facet not reported in previous studies: a kind of pit dimorphism. The larger pits clearly lack pit membranes, and we have no reason to believe that this membrane absence is an artifact. The scalariform perforation plate in Fig. 13 is noteworthy in that the bars are very slender, the perforations are correspondingly large, and some bars fork or anastomose. The facet at left in Fig. 13 bears intact pit membranes with the exception of two pits, which seem to lack pit membranes, probably a result of handling. The scalariform perforation plate of Fig. 14 has both wide bars and wide perforations and is the kind of perforation plate that could be seen with light microscopy. The other facets of the wide tracheary element in Fig. 14 lack pitting, a condition also shown for *Marsilea* vessels by Loyal and Singh (1978, Figs. 23 and 25). The slender tracheary element at right in Fig. 14 may be a late protoxylem or early metaxylem element; in any case, the facet at left bears intact pit membranes, whereas the facet at right has porose pit membranes like the ones shown in Fig. 8.

In *M. vestita,* a large tracheary element with very narrow elongate pits (Fig. 15) was found in the rhizome; no perforations were observed on this element. Scalariform perforations were observed on other rhizome tracheary elements (Fig. 16, left), and root tracheary elements (Fig. 17, right; porose pit membranes at bottom of perforation plate and intact pit membranes above perforation plate). The facet at right in Fig. 16 would be termed transitional in dicotyledons; this pitting type has apparently not been previously reported in *Marsilea.*

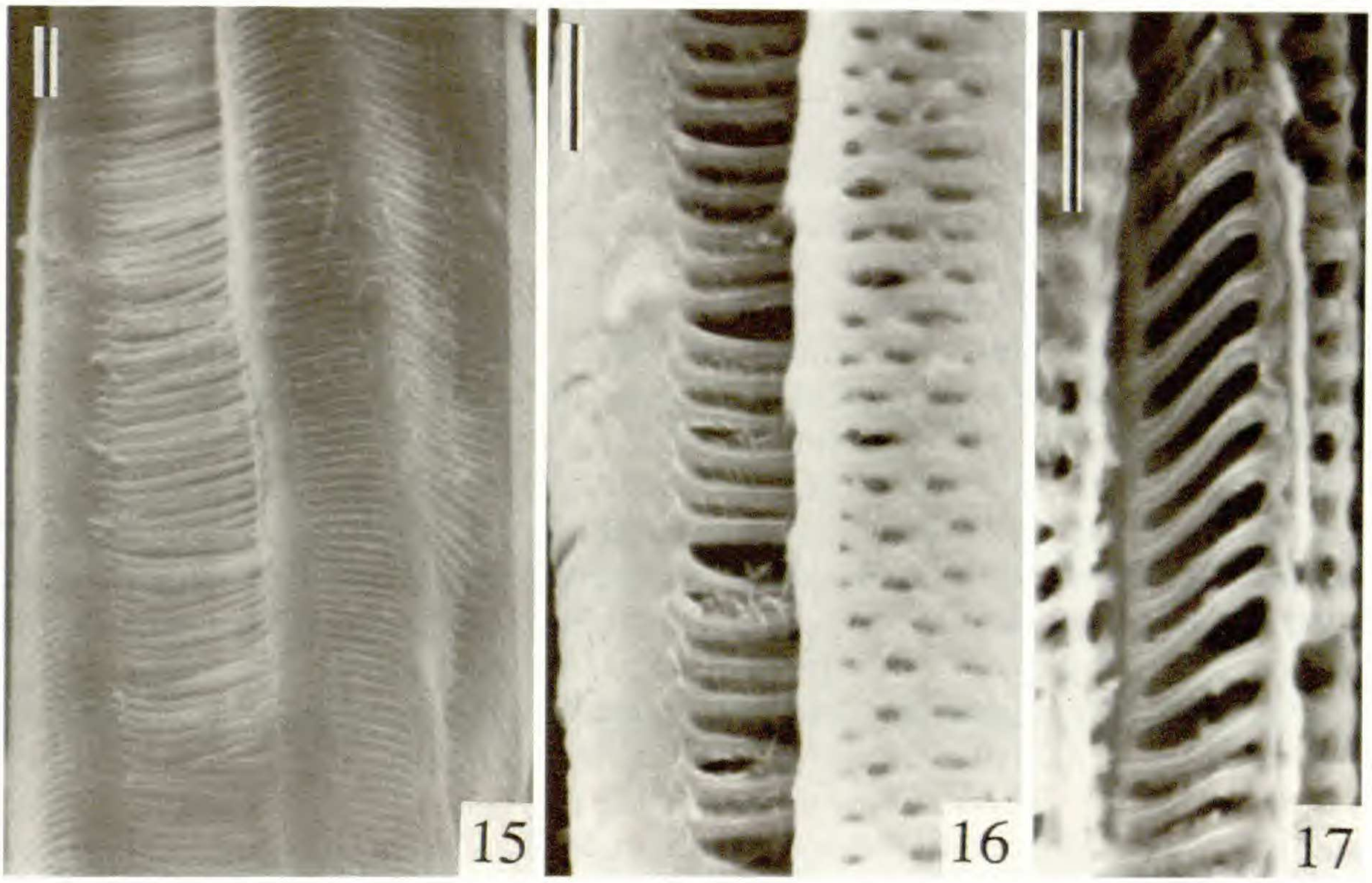
Discussion and Conclusions

Our investigation revealed features that have not previously been reported in tracheary elements of *Marsilea.* Among the features newly reported are pit dimorphism; transitional pits on lateral walls; and scalariform perforation plates with perforations like lateral wall pits in size and shape.

The pit dimorphism we report consists of wide pits, which lack pit membranes and are therefore perforations, that alternate with one or more extremely narrow pits that have pit membranes. This feature has not been reported to any appreciable extent before SEM studies, although Bierhorst (1960) figures pit dimorphism in *Asplenium.* We have found pit dimorphism (resulting in



FIGS. 11–14. SEM photographs of tracheary elements from rhizomes of *Marsilea quadrifolia*. 11) Portions of two tracheary elements; element at left bears scalariform perforation plates with minimal pit membrane remnants; element at right bears alternate pitting (some larger perforations appear to be perforations). 12) Portions of two tracheary elements; element at left has pit dimorphism, the larger openings are perforations; element at right has a scalariform perforation plate with pit membrane remnants in some perforations. 13) A well demarcated perforation plate (center) with slender bars, some of which anastomose. 14) Portions of two tracheary elements; perforation plates with wide perforations, left; slender element at right has porosities in pit membranes of right facet, but left facet has intact pit membranes. Scales in all figures = 5 μm.



FIGS. 15–17. SEM photographs of tracheary elements from rhizome (Figs. 15, 16) and root (Fig. 17) of *Marsilea vestita*. 15) Wide tracheary elements with linear scalariform pitting on each of three facets. 16) Scalariform pitting with porose pit membranes in facet at left; facet at right bears transitional pitting. 17) Scalariform perforation plate; several pits with intact membranes at top, several porose pit membranes at bottom of plate. Scales in all figures = 5 μm.

perforation plates) in a number of fern families and genera, such as *Phlebodium* (Schneider and Carlquist, 1997), *Anemia* (Carlquist and Schneider, 1998), *Angiopteris* and *Danaea* (Carlquist and Schneider, 1999).

Loyal and Singh (1978) have figured alternately arranged circular to oval lateral wall pits on *Marsilea* vessels, such as we have illustrated in *M. quadrifolia* rhizomes, but our report of transitional pitting (similar to opposite pitting) in *M. vestita* rhizomes is apparently a new report in ferns. Likewise, we have observed unpitted facets on *Marsilea* vessels, as have Loyal and Singh (1978). Unpitted facets in *Marsilea* vessels may face fibers, which sheathe vascular strands in the genus, but macerations cannot offer a clear interpretation. This matter should be studied, particularly because unpitted facets on tracheary elements have not been reported in ferns other than *Marsilea*.

Simple perforation plates and perforation plates with few bars have been reported for *Marsilea* by several authors (White 1961, 1962; Mehra and Soni, 1971; Bhardwaja and Baijal, 1977; Loyal and Singh, 1978). Our material did not yield vessel elements with simple perforation plates, but we did observe a perforation plate traversed by a single bar. The maceration techniques used for our SEM studies did, however, reveal scalariform perforation plates similar to pitting on the lateral walls of tracheary elements. Loyal and Singh (1978) have figured a few scalariform perforation plates with numerous bars in *Mar-*

silea, and those perforation plates are rather unlike lateral wall pitting in morphology, and are therefore likely to be seen with light microscopy. Our finding of perforation plates similar to scalariform lateral wall pitting is not unexpected in view of the Loyal and Singh (1978) findings, but in addition, perforation plates that are similar in secondary wall framework to lateral wall pits, may have escaped notice by previous workers who worked with light microscopy. Perforation plates that resemble lateral wall pitting in size and shape of the perforations are common in many ferns, according to our studies, so the occurrence of such perforation plates in *Marsilea* is not surprising.

Our material did not yield vessel elements with simple perforation plates. When one views intact stele portions with light microscopy, tracheary elements can be seen even when they are sheathed by fibers. In such preparations, simple perforation plates can be located more readily (e.g., White, 1962). *Marsilea* is unique among ferns in having simple perforation plates. Perforation plates with a few bars do occur in *Marsilea* but also in some other ferns, such as *Pteridium* (Carlquist and Schneider, 1997a) and *Astrolepis* (Carlquist and Schneider, 1997b). The ecological nature of ferns with such vessel elements is illuminating: all grow in areas with marked extremes of moisture availability and/or temperature. Both of these factors make water available for only a short season. Although *Marsilea* is commonly thought to be an aquatic fern, it can grow in temporary ponds in which water is available for only a relatively brief portion of the year. Simple perforation plates and perforation plates with few bars potentially offer the least resistance to rapid flow of a given volume of water per unit time, and the occurrence of such perforation plates in vessels (especially root vessels) of plants (especially monocotyledons) that live in habitats with brief wet seasons (Carlquist, 1975) is understandable.

Literature Cited

BHARDWAJA, T. N., and J. BAIJAL. 1977. Vessels in rhizome of *Marsilea*. Phytomorphology 27:206–208.

BHARDWAJA, T. N., and T. TAKKER. 1979. Tracheary elements of *Pilularia*. Phytomorphology 29: 388–389.

BIERHORST, D. W. 1960. Observations on tracheary elements. Phytomorphology 10:249–305.

CARLQUIST, S. 1975. *Ecological strategies of xylem evolution*. University of California Press, Berkeley and Los Angeles.

——— and E. L. SCHNEIDER. 1997a. SEM studies on vessels in ferns. 2. *Pteridium*. Am. J. Bot. 84: 581–587.

——— and E. L. SCHNEIDER. 1997b. SEM studies on vessels in ferns. 4. *Astrolepis*. Am. Fern J. 87: 43–50.

——— and ———. 1998. SEM studies on vessels in ferns. 10. Selected Osmundaceae and Schizaeaceae. Int. J. Plant Sci. 159:788–797.

——— and ———. 1999. SEM studies on vessels in ferns. 12. Marattiaceae, with comments on vessel patterns in eusporangiate ferns. Am. J. Bot. 86:457–464.

JOHANSEN, D. A. 1940. *Plant microtechnique*. McGraw Hill, New York.

JOHNSON, D. M. 1986. Systematics of the New World species of *Marsilea* (Marsileaceae). Syst. Bot. Monogr. 11:1–87.

LOYAL, D. S., and H. SINGH. 1978. A further investigation of the morphology of vessels in *Marsilea*. Proc. Indian Acad. Sci. (Plant Sciences-4) 87B:335–346.

MEHRA, P. N., and S. L. SONI. 1971. Morphology of tracheary elements in *Marsilea* and *Pteridium*. Phytomorphology 21:68–71.

SHARMA, B. D. 1988. Tracheary elements in pteridophytes. Bionature 8:113–134.

SCHNEIDER, E. L., and S. CARLQUIST. 1997. SEM studies on vessels in ferns. 3. *Phlebodium* and *Polystichum*. Int. J. Plant Sci. 158:343–349.

TEWARI, R. B. 1975. Structure of vessels and tracheids of *Regnellidium diphyllum* Lindm. (Marsileaceae). Ann. Bot., n.s., 39:229–231.

WHITE, R. A. 1961. Vessels in roots of *Marsilea*. Science 133:1073–1074.

———. 1962. A comparative study of the tracheary elements of the ferns. Ph.D. dissertation, University of Michigan, Ann Arbor.